REPORT DOCUMENTATION PAGE

Dist A

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
		ANNUAL 01 Jun 93 TO 31 May 94

4. TITLE AND SUBTITLE
AASERT-92 AUGMENTATION OF RESEARCH TRAINING IN CHRONO-BIOLOGY: REGULATION OF THE MAMMALIAN CIRCADIAN CLOCK BY NEUROTRANSMITTERS

5. FUNDING NUMBERS
F49620-93-1-0413
61103D
3484/YS

6. AUTHOR(S)
Dr Martha Gillette

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Dept of Cell & Structural Biology
Univ of Illinois
506 Morrill Hall, 505 South Goodwin Avenue
Urbana IL 61801

8. PERFORMING ORGANIZATION REPORT NUMBER
AFOSR-TR- 94 0681

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AFOSR/NL
110 Duncan Ave Suite B115
Bolling AFB DC 20332-0001

Dr Haddad

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

Accesion For
NTIS CRA&I [X]
DTIC TAB [ ]
Unannounced [ ]
Justification
By
Distribution/

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release.
Distribution unlimited.

DISTRIBUTION STATEMENT A
Approved for public release;
Distribution Unlimited

A

12b. DISTRIBUTION CODE

Availability Codes

Dist	Avail and/or Special
A-1	

13. ABSTRACT *(Maximum 200 words)*

Our research program aims to understand the mechanisms by which major neurotransmitter pathways regulate the biological clock in the suprachiasmatic nucleus (SCN) of the mammalian brain. Our model species is the rat. The specific progress made by each of the three students supported by ths AASERT award in FY1 is summarized below. Each of these students has maintained satisfactory grades and progress toward their degree requirements during the funding period.

14. SUBJECT TERMS

15. NUMBER OF PAGES

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
(U)	(U)	(U)	(U)

AASERT Technical Report

(FY92 AASERT) AUGMENTATION OF RESEARCH TRAINING IN CHRONOBIOLOGY: REGULATION OF THE MAMMALIAN CIRCADIAN CLOCK BY NEUROTRANSMITTERS

Grant No. F49620-93-1-0413

Martha U. Gillette, P.I.
Dept. of Cell & Structural Biology, University of Illinois, U-C

Our research program aims to understand the mechanisms by which major neurotransmitter pathways regulate the biological clock in the suprachiasmatic nucleus (SCN) of the mammalian brain. Our model species is the rat. The specific progress made by each of the three students supported by this AASERT award in FY1 is summarized below. Each of these students has maintained satisfactory grades and progress toward their degree requirements during the funding period.

STEVEN M. DEMARCO: GAD is the key biosynthetic enzyme for GABA, a major inhibitory neurotransmitter in the SCN. Steve examined the hypothesis that the concentration and specific activity of glutamic acid decarboxylase (GAD) in SCN are under circadian clock regulation. By probing Western blots of SCN proteins separated by polyacrylamide gel electrophoresis with antibodies specific to the two major isoforms, Steve demonstrated that both GAD_{65} and GAD_{67} are expressed in the SCN. They are present throughout the diurnal cycle, at circadian times (CTs) 4,10,16 and 22, in nearly equal concentrations. These isoforms paralleled each other in spontaneous circadian changes in abundance: They were high daily at CTs 10 and 22, and low at CT 16 (Tukey 1-way ANOVA, $p \leq .005$). Specific activity was evaluated at eight points in the 24-h cycle. Pyrodoxyl phosphate (PLP)-stimulated activity exhibited significant highs at CT 10 and 19 ($p \leq .04$), where as this cofactor-stimulated activity expressed a significant low at CT 4. These results suggest that there is circadian modulation of GAD activity, which is partially under the control of the cofactor. Interestingly, levels and activity both peaked in late day and late night. These two times may represent different inhibitory states for the SCN, one acting within neurons and circuits within this structure (CT 10), and another acting external to inhibit efferent targets as the circadian system moves into the inactive period for this nocturnal rodent (CT 19/22). Steven DeMarco was awarded his M.S. in Biology from the University of Illinois in May, 1994 and has joined a neuroimmunology doctoral program at the Mayo School for Graduate Research, Rochester, MN.

MARIJA MEDANIC: Marija has continued her investigation of the regulatory role of serotonin (5HT) and neuropeptide Y (NPY) upon the phasing of the SCN clock. Information from other brain regions to SCN carried by these neurotransmitter systems is thought to convey information about photic changes and behavioral arousal states during the daytime portion of the cycle. Afferents from the raphe (5HT) and intergeniculate leaflet (NPY) terminate in ventrolateral SCN, often upon the same neurons. Both 5HT and NPY induce phase advance of SCN rhythms in daytime when applied alone. To investigate the hypothesis that integration of nonphotic modulatory signals occurs directly at the level of the SCN, Marija tested that ability of 5HT and NPY to alter the phase of the SCN when applied simultaneously to the SCN *in vitro.* Potential interactions were evaluated at CT 7 and CT 23, both points of sensitivity of the SCN to NPY.

SCN brain slices were treated with microdrops (10^{-11} ml) containing a fresh mixture of 5HT and NPY. The concentration of 5HT was kept constant at 10^{-6} M, while varying the concentration of NPY. The effects of these treatments on the phase of the rhythm of electrical activity of the population of SCN neurons were assessed on the second day *in vitro.* While equimolar concentrations of NPY and 5HT caused phase advances of 3.5 ± 0.2 h, the same phase shift as NPY alone, decreasing the NPY concentration resulted in the larger phase shifts that are characteristic of 5HT alone at CT 7. At CT 23, the shift was characteristic of NPY alone, with no effect of 5HT. This demonstrates that putative neurotransmitters for nonphotic zeitgebers can interact directly at the level of the SCN.

THOMAS K. TCHENG: Tom is continuing his efforts to develop a multiunit electrode that can simultaneously monitor the activity of a small population of SCN neurons. He has succeeded in modifying our usual brain slice system into one that automatically records the circadian oscillation in neuronal firing rate for at least 3 and more often 4-5 days *in vitro.* He has developed the computer software to acquire, store and analyze this data on line. He has used this system to compare the period of the free-running SCN circadian rhythm of neuronal activity with free-running behavioral rhythms in locomotory activity, drinking and temperature rhythms, which were measured in the behavioral monitoring system in Dr. Evelyn Satinoff's laboratory. He has found that the period of the SCN rhythm in the brain slice, which contains less than the entire SCN, matches very closely the organismic rhythms measured in freely behaving animals from our inbred rat colony. He is using this recording system to assess the hypothesis that glutamate, the putative neurotransmitter carrying information about light signals from the optic nerves to SCN, induces both acute and long-term effects upon groups of SCN neurons.